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THE EFFECT OF LIPID COMPOSITION OF SMALL UNILAMELLAR LIPOSOMES CONTAINING MELPHALAN AND VINCRISTINE ON DRUG CLEARANCE AFTER INJECTION INTO MICE*

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Abstract—Melphalan and vincristine together with their radiolabelled derivatives were entrapped in small unilamellar liposomes of varying choleresterol content and phospholipid composition. After intravenous injection of drug-containing egg phosphatidylcholine liposomes into mice, drug clearance rates from the blood were reduced with increasing cholesterol content. Circulating drugs were partially associated with the carrier and partly free, mostly bound to plasma proteins. The ratio of drug associated with liposomes to that circulating as free was dependent on the type of liposomes used and highest when these were cholesterol-rich. Drug clearance rates were reduced and entrapped:free drug ratios increased further when egg phosphatidylcholine in cholesterol-rich liposomes was replaced by sphingomyelin. Drug-containing cholesterol-rich liposomes injected intraperitoneally were found capable of entering the periphery intact and quantitatively to assume clearance rates similar to those observed after intravenous treatment. Such manipulations in liposomal lipid composition can alter pharmacokinetics in ways that could provide optimal conditions for drug distribution into tumours and a therapeutic effect.

The possibility of a role for liposomes in the use of anti-cancer drugs has aroused considerable interest in recent years [1, 2]. Two distinct areas of potential usefulness have been suggested: as carriers for the direct delivery of drugs to tumour cells or, when these are not accessible to liposomes, as reservoirs for the release of drugs at predetermined rates. The latter is especially applicable to phase-specific drugs for which therapeutically effective concentrations in the plasma during the target cell's cycle are required. It has been recently shown that cholesterol incorporated into liposomes composed of egg phosphatidylcholine (PC), dimyristoylphosphatidylcholine (DMPC), sphingomyelin (SM) and other phospholipids reduces in proportion to its concentration the release of entrapped model solutes in the presence of blood *in vitro* at 37° [3–8] and in the intravascular space *in vivo* [3, 4, 9–11]. Cholesterol acts by preventing [7, 8, 12] liposomal phospholipid loss to high-density lipoproteins (HDL) and ensuing pore formation [13] in the bilayers. Furthermore, the half-life of liposomes and entrapped solutes in the circulation of injected animals can be modified by the appropriate choice of the liposomal phospholipid component [9–11]. Phospholipids determine liposomal half-life by the extent to which, either alone or in association with cholesterol, they prevent plasma-induced bilayer permeability [11]. In view of these findings, it seemed feasible that appropriate manipulation of the lipid composition of liposomes containing cytotoxic drugs could allow adjustment of the pharmacokinetic behaviour of drugs in the circulation so as to provide optimal conditions for their localization in tumours (following release from the carrier) and action. In this report and in one to follow‡ we have examined the effect of the liposomal cholesterol content and phospholipid component on plasma clearance of the phase-specific drugs melphalan and vincristine, their distribution in the tissues of tumour-bearing mice and their therapeutic effect. Neutral, small unilamellar liposomes were used throughout, since these are known [4, 14, 15] to circulate in the bloodstream longer than larger vesicles or negatively charged vesicles of the same size.

MATERIALS AND METHODS

Sources and grades of egg L-α-PC, L-α-DMPC, SM, cholesterol, Ultrogel AcA 34 and Sepharose CL 6B have been described elsewhere [9, 10]. Vincristine sulphate (Oncovin) and melphalan (Alkeran) were kindly donated by Lilly Research Centre Ltd (Surrey, U.K.) and Burroughs Foundation Ltd (Kent, U.K.) respectively. [G-^{3}H]Vincristine sulphate (sp. act. 4.2 Ci/mmole) was from the Radiochemical Centre (Amersham, U.K.) and ^{14}C-labelled melphalan (labelled in the ethyl side chains, sp. act. 12.35 mCi/mmole) was a gift from S.R.I. International (CA, U.S.A.). All other reagents were of analytical grade.

Preparation of liposomes. Drug-containing small

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unilamellar liposomes (30–60 nm diameter) were prepared [4, 9, 10] from 20 μmoles phospholipid/ml of final preparation, supplemented where needed with the appropriate amount of cholesterol. The lipids were dried by rotary evaporation of the organic solvent to a thin film on the walls of a 50-ml round-bottomed flask, and any traces of solvent removed under a stream of nitrogen. Lipids were then dispersed by shaking with 1.3 ml phosphate-buffered saline (PBS) (pH 7.4) composed of 0.8% NaCl, 0.02% KCl, 0.32 mM $NaH_2PO_4.2H_2O$ and 0.14 mM $Na_2HPO_4.2H_2O$ and containing 1.4–1.8 mg melphalan or 24–30 μg vincristine and their radiolabelled derivatives (32.5 μCi [^{14}C]melphalan and 11.7 μCi [^{3}H]vincristine). Prior to its use, melphalan was dissolved in 30 μl of Alkeran solvent made up with 0.94 ml HCl (36% w/w) and 19 ml ethanol (96% v/v) and the solution diluted to 1.3 ml with PBS. The preparations were sonicated [4, 9, 10] for 10 min (1-min sonication with 30-sec cooling periods), allowed to stand at room temperature for 1 hr, and then centrifuged at 100,000 *g* for 60 min to remove larger multilamellar structures and membrane fragments. Drug-containing small unilamellar liposomes in the supernatant were separated from non-entrapped drug on a Sepharose CL 6B column (20 × 0.8 cm, total bed volume 10 ml) equilibrated with PBS. Liposomes were eluted in 1.5–2.0 ml in the void volume and then dialysed overnight at 4° against the same buffer before use. Drug entrapped was estimated from assays [16] of ^{14}C and ^{3}H values in the final liposomal preparations.

In vivo *experiments*. Free and liposome-entrapped radiolabelled melphalan or vincristine were injected intravenously via the tail vein (0.25 ml or 2.2–2.8 mg phospholipid injected) or intraperitoneally (0.35 ml or 3.2–3.8 mg phospholipid injected) into T.O. mice weighing 25–30 g. The dosages of injected drugs ranged from 200–500 μg/kg for melphalan (4.4×10^4–4.1×10^5 dpm) and 20–100 μg/kg for vincristine (5.5×10^4–2.3×10^6 dpm). Following treatment, animals were bled from the tail at time intervals. Heparinized blood samples (25 or 50 μl) were then mixed with 1 ml PBS, centrifuged at 1000 *g* for 5 min to pellet the cells and the supernatants assayed for ^{14}C and ^{3}H radioactivity [16]. No attempts were made to distinguish between parent drug and any radioactive metabolic products that might be formed. In other experiments, plasma from 0.1 ml of blood samples obtained after injection of liposomes was chromatographed on Ultrogel AcA 34 columns (20 × 0.8 cm, total bed volume 10 ml) and 0.5-ml fractions collected were assayed for ^{14}C and ^{3}H radioactivity [16].

In vitro *experiments*. Fresh heparinised T.O. mouse blood was obtained by exsanguination from the axilla and the blood cells pelleted by centrifugation and discarded. Plasma (1 ml) was then mixed with 0.2 ml liposomes containing radiolabelled melphalan (10^4–1.6×10^4 dpm) or vincristine (4×10^4–1.2×10^5 dpm) and incubated at 37°. At time intervals, aliquots (0.3-ml) of the incubation mixtures were removed and chromatographed on Ultrogel AcA 34 columns (22 × 1.1 cm, total bed volume 20 ml). Fractions (1-ml) collected were assayed as earlier for ^{14}C or ^{3}H radioactivity.

RESULTS AND DISCUSSION

Entrapment of melphalan and vincristine in liposomes of varying lipid composition

Judging from radioactivity values in Table 1, entrapment of either drug in PC liposomes was highest when cholesterol was equimolar with the phospholipid (cholesterol-rich liposomes). Much more vincristine was entrapped (14.4%) than melphalan (3.0% of the amount used) and, clearly, incorporation of vincristine in liposomes increased in proportion to their cholesterol content. For cholesterol-rich SM liposomes, vincristine entrapment values were even higher (39.5%) whereas those of melphalan remained low (3.1%). Such high percentage values of vincristine entrapment cannot be justified on the basis of drug inclusion into the aqueous space of the small unilamellar liposomes used in this study. It is, therefore, likely that much of the liposome-associated vincristine partitions into the lipid bilayers in the course of their formation. Once formed, bilayers would be expected to retain the drug because of the cholesterol-induced packing

Table 1. Incorporation of melphalan and vincristine into liposomes of varying lipid composition*

Liposomes	Phospholipid : cholesterol molar ratio	Entrapment (% + S.E.)	
		Melphalan	Vincristine
PC		1.9 ± 0.2 (3)	1.8 ± 0.1 (3)
PC, C	1:0.2	2.0	2.4
	1:0.4	2.0	2.5
	1:0.6	1.7 ± 0.2 (3)	6.3
	1:0.8	1.9 ± 0.0 (3)	7.5
	1:1	3.0 ± 0.4 (3)	14.4 ± 0.9 (3)
DMPC, C	1:1	2.4	13.0 ± 2.3 (3)
SM, C	1:1	3.1	39.5 ± 3.3 (3)

* [^{14}C]Melphalan and [^{3}H]vincristine were incorporated into small unilamellar liposomes containing various amounts of cholesterol (C) as described in Materials and Methods. Entrapment values are % ± S.E. of total drug used. Numbers in parentheses denote numbers of preparations.

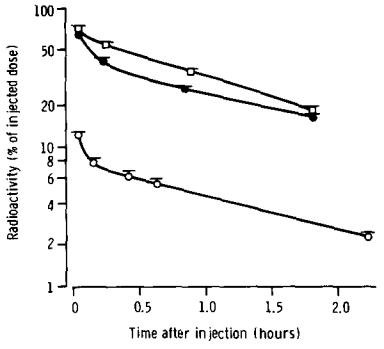

Fig. 1. The effect of the cholesterol content of liposomes containing [^{14}C]melphalan on drug clearance from the blood of intravenously injected mice. Melphalan was administered intravenously in the free form (○) or entapped in cholesterol-free (●) or cholesterol-rich (□) PC liposomes. Mice were bled from the tail vein at time intervals and plasma analysed for radioactivity. Values (means from four animals) are expressed as % ± S.E. of the injected dose per total mouse plasma [4].

of phospholipid molecules [17, 18]. Furthermore, since vincristine is more lipophilic than melphalan it may be that the former, used in much smaller amounts [24–30 μg) than the latter (1.4–1.8 mg), partitions into the bilayers more efficiently.

Drug clearance after intravenous injection of melphalan and vincristine entrapped in liposomes of varying cholesterol content

We have studied the effect of cholesterol presence in PC liposomes containing melphalan or vincristine on drug clearance from the blood of injected animals. The choice of PC as the liposomal phospholipid component was based on previous experience [3, 4, 9–13] with similar liposomes containing carboxyfluorescein as a model solute. Figs 1 and 2 show that the rapid (<5% of injected radioactivity retained in blood after 1 hr) clearance of both radiolabelled drugs administered as free is reduced considerably (25% retained) by their previous entrapment in cholesterol-free liposomes. Clearance rates are further reduced (35% retained) when drugs are entrapped in cholesterol-rich liposomes incorporating equimolar cholesterol. This cholesterol-promoted reduction in clearance rates was, at least in the case of vincristine, measurable only above a certain phospholipid : cholesterol molar ratio [1 : 0.4 (Fig. 2)] and did not appear to be as great as when carboxyfluorescein was the entrapped solute (5 and 65% of the injected dye retained in blood after 1 hr for cholesterol-free and cholesterol-rich liposomes respectively [4]). Furthermore, whereas clearance of carboxyfluorescein administered in cholesterol-rich liposomes was linear [4, 9–11], i.e. identical to that of its carrier, clearance of melphalan and vincristine entrapped in similar liposomes was biphasic showing a rapid initial phase followed by a prolonged linear one (Figs 1 and 2). In contrast to the highly polar carboxyfluorescein, melphalan and vincristine are lipophilic and therefore likely [19] to leak through the bilayers. This and the anticipated [20, 21] binding of the drugs already leaked into the circulation to plasma proteins would be expected to influence overall clearance rates considerably.

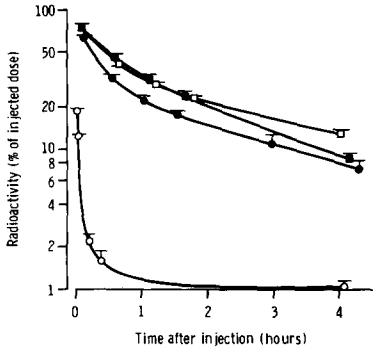

Fig. 2. The effect of the cholesterol content of liposomes containing [^{3}H]vincristine on drug clearance from the blood of intravenously injected mice. Vincristine was administered intravenously in the free form (○) or entrapped in PC liposomes of varying cholesterol content: (●) cholesterol-free and cholesterol-containing (molar ratio 1 : 0.2) liposomes; (■) cholesterol-containing liposomes (molar ratios of 1 : 0.4 and 1 : 0.6); (□) cholesterol-containing liposomes (molar ratios of 1 : 0.8 and 1 : 1). Values (means from eight animals, four for each of the two liposome types) of each of the pairs of liposomal preparations were pooled because of similarity and are expressed as % ± S.E. of the injected dose per total mouse plasma.

Drug clearance after intravenous injection of melphalan and vincristine entrapped in liposomes with varying phospholipid component

It has been already reported [9–11] that the choice of phospholipid component in preparing small unilamellar liposomes can profoundly influence the rate of clearance of entrapped solutes from the circulation. For instance, carboxyfluorescein entrapped in cholesterol-rich liposomes composed of PC, DMPC, SM or distearoylphosphatidylcholine exhibits in injected mice linear rates of clearance with half-lifes of 2, 6, 16 and 20 hr respectively [9–11]. In the present studies PC, DMPC and SM were used to prepare cholesterol-rich liposomes containing radiolabelled melphalan or vincristine. Results in Fig. 3 show that, as with carboxyfluorescein [9–11], drugs administered in SM liposomes remain in the circulation of intravenously injected mice for a considerably longer period than when given in PC or DMPC liposomes. However, whereas carboxyfluorescein clearance was linear throughout for all three types of liposomes [9–11], those of the two drugs were biphasic with a rapid initial phase followed by a slower linear one (Fig. 3). Furthermore, and in contrast to the behaviour of carboxyfluorescein [9, 10], drug clearance rates (Fig. 3) for PC and DMPC liposomes were nearly identical (for a possible explanation see later).

Drug clearance after intraperitoneal injection of vincristine entrapped in liposomes with varying phospholipid component

Previous work [4, 22] has shown that, after intraperitoneal injection of liposome-entrapped carboxyfluorescein, the dye enters the circulation quantitatively via intact liposomes. As treatment of

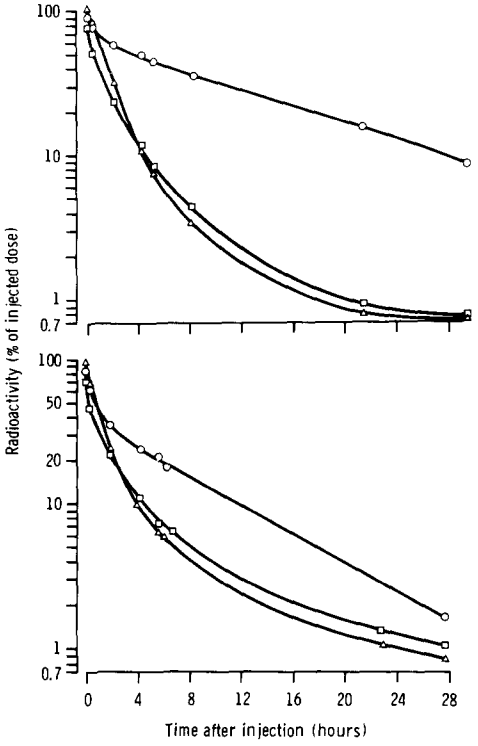

Fig. 3. The effect of phospholipid composition of liposomes containing [^{14}C]melphalan or [^{3}H]vincristine on drug clearance from the blood of intravenously injected mice. Cholesterol-rich PC (△), DMPC (□) and SM (○) liposomes containing [^{14}C]melphalan (upper) or [^{3}H]vincristine (lower) were injected intravenously into mice which were then bled at time intervals. Plasma was analysed for radioactivity and values (means from four animals) are expressed as % of the injected dose per total mouse plasma. S.E., being less than 4%, are not shown.

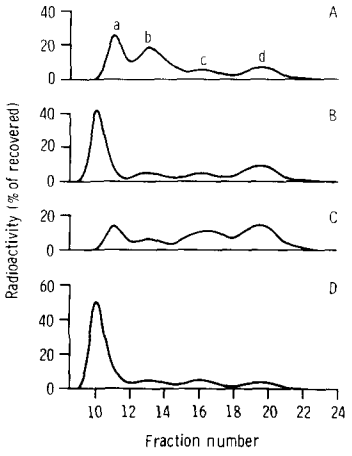

Fig. 5. The effect of liposomal cholesterol on drug release from PC liposomes during incubation with mouse plasma. Cholesterol-free (A and C) and cholesterol-rich (B and D) liposomes containing [^{3}H]vincristine (A and B) or [^{14}C]melphalan (C and D) were incubated with mouse plasma at 37°. After 2.5 min, samples were removed and chromatographed on Ultrogel AcA 34 and fractions collected analysed for radioactivity. Values in each of the peaks obtained are expressed as % of total radioactivity recovered from the columns. Recoveries were 95–106% of the quantities applied. For other details see Materials and Methods.

experimental tumours is often carried out through the intraperitoneal route, it was of interest to see whether liposomal drugs behave similarly to liposomal carboxyfluorescein and, if so, to study their rates of clearance from the circulation. Results in Fig. 4 show that after intraperitoneal injection of mice with cholesterol-rich PC, DMPC or SM liposomes containing radiolabelled vincristine, patterns of drug entry into the circulation are very similar for the three preparations, each showing a peak of blood radioactivity (24.1–29.5% of the dose) at 2 hr. This compares to a peak concentration of 1.4% at 9 min for the free drug (Fig. 4) of which the rate of entry into the blood is, apparently, lower than its rate of clearance. On the other hand, patterns of clearance of liposomal vincristine from the circulation (Fig. 4) differed according to the phospholipid component and were similar to those observed after intravenous injection (Fig. 3). Clearance rates were, however,

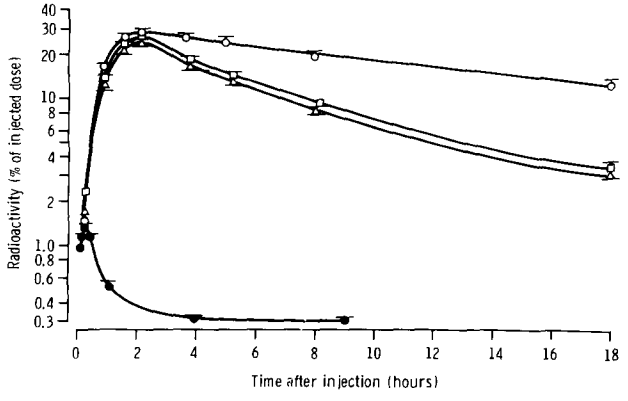

Fig. 4. The effect of phospholipid composition of liposomes containing [^{3}H]vincristine on drug clearance from the blood of intraperitoneally injected mice. Vincristine in the free form (●) or entrapped in cholesterol-rich PC (△), DMPC (□) or SM (○) liposomes was injected intraperitoneally into mice which were then bled at time intervals. Plasma was analyzed for radioactivity and values (means from four animals) are expressed at % ± S.E. of injected dose per total mouse plasma.

reduced presumably because of a more rapid liposomal drug entry into the blood than exit from it (Fig. 4).

Effect of the lipid composition of liposomes on the release of entrapped melphalan and vincristine in the presence of plasma

In an attempt to clarify some of the data obtained *in vivo* (Figs 1–4), cholesterol-free PC and cholesterol-rich PC, DMPC and SM liposomes containing radiolabelled vincristine or melphalan were incubated with mouse plasma at 37° and aliquots removed at time intervals (up to 4 hr to take into account the prolonged presence of SM liposomes in the circulation *in vivo*) fractionated by molecular sieve chromatography. Fig. 5 shows drug elution profiles after incubation of cholesterol-free and cholesterol-rich PC liposomes for 2.5 min. With each type of liposomes (both drugs), portions of the radiolabel are eluted with the vesicles in peak a corresponding to the void volume and as three additional peaks, peak d representing released free drug and peaks b and c representing released drug probably bound to plasma proteins [20, 21] [indeed, the involvement of plasma in drug release from liposomes and appearance in peaks b and c was supported by the finding that after incubation with PBS more than 95% of the radioactivity is recovered with peak a and none with peaks b and c (results not shown)]. Moreover, radiolabel distribution between peaks b and c is non-uniform, especially where increased drug release has occurred (Fig. 5A and C), with vincristine eluting preferentially in peak b and melphalan in peak c. Many lipophilic anti-cancer drugs bind non-specifically to plasma proteins, especially albumin. Vincristine, however, is unusual in that it binds more strongly to α- and β-globulins [20]. It is likely, therefore, that peak b corresponds to fractions where these globulins are eluted, and that peak c fractions are associated with some other component (e.g. albumin).

Quantitation of plasma-induced drug release from these liposomes (Table 2) showed that, as with carboxyfluorescein [4], drug loss from cholesterol-free liposomes was rapid and pronounced [e.g. 82.5% total melphalan released after 2.5 min as peaks b–d (Table 2)]. At both time intervals studied (2.5 and 65 min) much of the released drugs was associated with peaks b and c, presumably bound to plasma proteins (Table 2). On the other hand, there was less total (peaks b–d) drug loss from cholesterol-rich liposomes even after 65 min incubation (43.1 and 58.6% melphalan and vincristine lost respectively) and, again, a considerable proportion was associated with peaks b and c (Table 2). However, such losses were much greater than those (less than 6%) observed under identical conditions for carboxyfluorescein [4]. Whilst it is conceivable that some type of drug–lipid interaction partially destabilises cholesterol-rich liposomes or renders them susceptible to attack by plasma HDL, it is more likely [23] that plasma proteins remove drug weakly adsorbed onto the liposomal surface. This could also account for some of the drug release seen with cholesterol-free liposomes (see earlier) although, in this case, bilayer destabilization by plasma HDL [6, 8, 12] is probably the main cause. Increased drug loss from liposomes soon after exposure to plasma can thus explain the rapid initial phase of drug clearance *in vivo* (Figs 1 and 2): following their release, both melphalan and vincristine are expected to rapidly diffuse into the extravascular space [24].

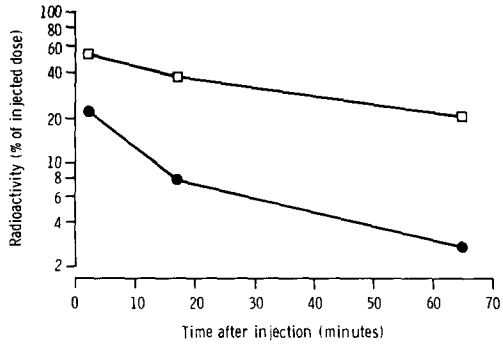

Fig. 6. Entrapped drug clearance after intravenous injection of mice with vincristine-containing liposomes. Cholesterol-free (●) and cholesterol-rich (□) PC liposomes containing [^{3}H]vincristine were injected intravenously into mice (three in each group). Animals were bled at time intervals and plasma samples chromatographed on Ultrogel AcA 34 columns. Radioactivity eluting in the fractions corresponding to the liposomal peak (peak a) was then estimated and expressed as % (mean) of the total radioactivity injected per total mouse plasma. Recoveries of radioactivity from the column were 96–99% of the quantities applied.

Table 2. Release of melphalan and vincristine from cholesterol-free and cholesterol-rich liposomes in the presence of plasma*

		2.5 min			65 min		
Drug	Liposomes	Peak b	Peak c	Peak d	Peak b	Peak c	Peak d
Melphalan	PC	14.1	29.9	38.5	15.5	32.5	45.0
	PC, C	10.0	10.7	16.1	12.4	13.7	17.0
Vincristine	PC	36.6	13.4	19.3	39.0	16.4	32.9
	PC, C	11.0	10.0	29.1	12.5	11.6	34.5

* Small unilamellar liposomes composed of PC only or equimolar PC and cholesterol (C) contained [^{14}C]melphalan or [^{3}H]vincristine. Liposomes were incubated in the presence of mouse plasma at 37° for 2.5 and 65 min and samples were subsequently chromatographed. Released ^{14}C and ^{3}H radioactivity in peaks b–d was then measured and expressed as % of total recovered. Recoveries were 94.6–99.1% of the radioactivity applied.

Table 3. Release of melphalan and vincristine from liposomes with varying phospholipid component in the presence of plasma

		10 min		4 hr	
Drug	Liposomes	Peaks b and c	Peak d	Peaks b and c	Peak d
Melphalan	PC, C	21.4	11.0	39.8	17.0
	DMPC, C	7.0	3.0	14.2	23.6
	SM, C	11.3	3.2	15.5	8.4
Vincristine	PC, C	21.4	29.7	29.4	47.5
	DMPC, C	14.6	13.5	25.7	44.8
	SM, C	6.0	11.5	7.2	37.1

* Small unilamellar liposomes composed of equimolar PC, DMPC or SM and cholesterol (C) contained [^{14}C]melphalan or [^{3}H]vincristine. Liposomes were incubated in the presence of mouse plasma at 37° for 10 min and 4 hr and samples were subsequently chromatographed as described in Materials and Methods. Released ^{14}C and ^{3}H radioactivity in peaks b–d was then measured and expressed as % of total recovered. Recoveries were 93.6–106.3% of the radioactivity applied.

It is also apparent from Fig. 5 and Table 2 that rates of drug clearance *in vivo* (Figs 1 and 2) are complex, representing those of entrapped and released (protein-bound or free) drug, both influenced in turn by the rate of drug dissociation or diffusion from the circulating vesicles. To estimate the extent to which liposomal cholesterol causes drugs to circulate in association with the carrier, cholesterol-free and cholesterol-rich liposomes containing [^{3}H]vincristine were injected intravenously into mice which were then bled at time intervals and blood plasma chromatographed. Liposomal vincristine values (peak a) in Fig. 6 are now free of interference from leaked or dissociated drug and reveal the considerable effect of cholesterol in reducing the rate of clearance of vincristine (compare with corresponding data in Fig. 2). It is conceivable that intermediate concentrations of cholesterol as used in Fig. 2 may lead to distinctly different intermediate rates of drug clearance in the entrapped form.

In agreement with results for (cholesterol-rich) PC liposomes (Fig. 5B and D), soon after mixing with plasma a significant proportion of drugs in DMPC and SM cholesterol-rich liposomes was released and eluted as peaks b–d (not shown). Furthermore, drug release [e.g. 28.1 and 17.5% total vincristine released after 10 min as peaks b–d from DMPC and SM liposomes respectively (Table 3)], although not as pronounced as with PC liposomes [51.1% (Table 3)], was still far greater than that measured for CF under similar conditions [9, 10] and could again explain the rapid initial phase (all three types of liposomes) of drug clearance *in vivo* (Fig. 3). It could also account to some extent for the similarity (Fig. 3) in drug clearance rates for PC and DMPC liposomes: any differences in entrapped drug clearance that could have existed *in vivo* may have been masked in a way similar to that discussed for cholesterol-free and cholesterol-rich liposomes (Figs 1, 2 and 6). Table 3 also shows that total release of vincristine exceeds that of melphalan probably because the greater lipophilicity of the former enables it to pass more readily through the lipid membranes. This is compatible with the finding (Fig. 3) that sphingomyelin liposomes show a more rapid overall clearance for vincristine than for melphalan.

In conclusion, incorporation of melphalan and vincristine into small unilamellar liposomes of varying cholesterol content and phospholipid composition can provide for a wide range of drug clearance rates from the circulation of injected animals (Figs 1–4). Because of the plasma-induced drug leakage or dissociation from liposomes (Tables 2 and 3), such rates are, at any time after injection, the sum of those exhibited by carrier-associated and free drug. In the latter case, a proportion is bound to plasma proteins (Fig. 5) which, presumably, also modify drug clearance to some extent [25]. It is thus possible that therapeutically optimal exposure of tumours (or of other target tissues) to drugs may be achieved by appropriately designed liposomes that can, as the case may be, either retain drug contents quantitatively for direct transport to accessible target cells or release drugs at predetermined rates. This will require clearer understanding of factors that govern both clearance of liposomes from the circulation and the concomitant release of the drugs they carry. Furthermore, in view of the observed differences in the behaviour of melphalan and vincristine in terms of association with, and release from liposomes, there will also be a need to tailor the carrier's lipid composition and other structural characteristics for individual drugs. In this respect it is encouraging that cholesterol-rich large liposomes containing cytosine arabinoside enhance the survival of ascites tumour-bearing mice more than liposomes devoid of, or poor in cholesterol [26, 27]. This is presumably due to the slow leakage of the phase-specific drug through the packed cholesterol-rich bilayers.

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